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Integrin αvβ1 Is a Receptor for Foot-and-Mouth Disease Virus

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Infection by field strains of *Foot-and-mouth disease virus* (FMDV) is initiated by binding to certain species of arginine-glycine-aspartic acid (RGD)-dependent integrin including αvβ3 and the epithelial integrin αvβ6. In this report we show that the integrin αvβ1, when expressed as a human/hamster heterodimer on transfected CHOB2 cells, is a receptor for FMDV. Virus binding and infection mediated by αvβ1 was inefficient in the presence of physiological concentrations of calcium and magnesium but were significantly enhanced by reagents that activate the integrin and promote ligand binding. The ability of chimeric α5/αv integrin subunits, in association with the β1 chain, to bind FMDV and mediate infection matched the ligand binding specificity of αvβ1, not α5β1, thus providing further evidence for the receptor role of αvβ1. In addition, data are presented suggesting that amino acid residues near the RGD motif may be important for differentiating between the binding specificities of αvβ1 and αvβ6.

Field strains of *Foot-and-mouth disease virus* (FMDV), the type species of the *Aphthovirus* genus of the *Picornaviridae* (3), infects cells by attaching to integrin receptors through a long surface loop, the GH loop of VP1 (22, 23, 24, 30, 33). The sequence of this loop contains a conserved tripeptide, arginine-glycine-aspartic acid (RGD), which is characteristic of the ligands of several members of the integrin family (20, 50). Integrins are cell surface α/β heterodimeric glycoproteins that contribute to a variety of functions, including cell-cell and cell-matrix adhesion and induction of signal transduction pathways (14, 16, 19, 20, 61).

A general property of integrins is that they exist in at least two conformations, active (competent to bind ligand) and inactive (unable to bind ligand) (50). Conversion from an inactive to an active state (integrin activation) is postulated to occur through two different mechanisms, collectively referred to as "inside-out signaling"; the first, avidity modulation, is mediated by clustering of heterodimers at the cell surface, whereas the second, affinity modulation, is mediated through conformational changes in the integrin ectodomain. Although the molecular mechanisms that regulate inside-out signaling in vivo remain unclear (14, 16, 19, 61), the conformational changes that occur naturally in the extracellular domains upon integrin activation can be induced experimentally by activating anti-integrin antibodies. These promote ligand binding by stabilizing epitopes that are expressed only on the active conformation (2, 39). The affinity of integrins for their ligands is also regulated by divalent cations (25, 37) and, in general, ligand binding is maximal in the presence of manganese ions, which are believed to stabilize shapes of the ligand binding pocket that favor ligand binding (27, 29).

Several viruses have been reported to utilize RGD-dependent integrins to initiate infection. Adenovirus has been shown to use αvβ3, αvβ5, and αvβ1 (28, 55, 56), and human parechovirus type 1 uses αvβ3 and αvβ1 (44, 52), whereas coxsackievirus A9 has been shown to use αvβ3 (45). In addition, αvβ1, αvβ3, and α5β1 have been implicated as receptors for coxsackievirus A9, the Barty strain of echovirus type 9, and adenovirus, respectively (13, 43, 44).

Since the various RGD-binding integrins have distinct tissue distributions, it is important to establish which species have the potential to act as receptors for FMDV. Prior to these studies, FMDV was reported to use two RGD-dependent integrins, αvβ3 and αvβ6, to initiate infection of cultured cells (4, 24), whereas the evidence for two other integrins, α5β1 and αvβ5, has been consistently negative (24, 32, 42). A fifth candidate integrin, αvβ1, has been difficult to study since its expression appears restricted in a cell-specific manner, as several cell types express both subunits in excess but do not appear to express this heterodimer (49, 53). In this report, we show that CHOB2 cells, which are normally nonpermissive for field strains of FMDV, become susceptible to infection after transfection with the integrin αv subunit, and we show by various criteria that this susceptibility is due to the expression of αvβ1 at the cell surface. Furthermore, we show that virus binding and infection mediated by αvβ1 are greatly enhanced in the presence of reagents that activate the integrin and promote ligand binding.

In addition, data are presented suggesting that amino acid residues near the RGD motif may be important for differentiating between the binding specificities of αvβ1 and αvβ6.

MATERIALS AND METHODS

Cells and viruses. Baby hamster kidney (BHK) cells, the α5-deficient Chinese hamster ovary (CHO) variant cell line CHOB2 (48), and stably transfected CHOB2 cell lines expressing αvβ6 (CHOB2-αvβ6) (54), αvβ1 (CHOB2-αvβ1) (38), or αv/α5 chimeras in association with the β1 subunit [αv/α5(F1-G232) and α5/αv(F1-G223), discussed in reference 38] were cultivated as described previously. The αv/α5(F1-G232) chimera consists of residues 1 to 232 of α5 followed by residue 224 of αv onwards and has an identical ligand binding specificity to wt (wild-type) α5β1 (38). The α5/αv(F1-G223) chimera consists of residues 1 to 223

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of αv followed by residue 233 of α5 onwards and has a ligand binding specificity identical to that of wt αvβ1 (38). Virus stocks of the FMDV strains, O1Kcad^2 and O1BFS, were prepared with primary bovine thyroid and BHK cells, respectively (24). The multiplicity of infection (MOI) (PFU per cell) values for both FMDV strains were based on the virus titer on BHK cells. Purification of FMDV was carried out as described previously (11).

Antibodies, peptides, and reagents. The GRGDSP and GRGESP peptides were purchased from Novabiochem. The FMDV VP1 GH loop peptide [FMDV-RGD (VPNLRGDLQVLA)], and the control RGE version (FMDV-RGE) were prepared as described previously (23). The anti-integrin monoclonal antibodies (MAbs) used in these studies were the functional blocking MAbs P1F6 (anti-αvβ5) and 10D5 (anti-αvβ6) from Chemicon, L230 (anti-αv), and the activating anti-β1 MAb 9EG7 (rat immunoglobulin G [IgG]) (2). The mouse MAb PB1, specific for hamster α5β1, was purchased from the Developmental Studies Hybridoma Bank (University of Iowa). MAb PB1 and the murine, anti-type-O FMDV MAbs, C9 (IgG2a) and B2 (IgG1) (34, 57), were purified with protein A (Pierce) according to the manufacturer's instructions. *R*-Phycoerythrin-conjugated antibodies were purchased from Southern Biotechnology Associates.

Flow cytometry analysis. (i) Standard assay. Flow cytometry was performed as described previously (36). Briefly, cells were harvested with EDTA and resuspended at ≈10^7 cells per ml in Tris-buffered saline (pH 7.4) containing 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 2% normal goat serum, and 3% bovine serum albumin (buffer A). Cells were incubated with primary antibodies (10 μg/ml in buffer A) on ice for 20 min followed by secondary antibodies conjugated with *R*-phycoerythrin. Background fluorescence was determined in the absence of the primary antibody. Fluorescent staining was analyzed by flow cytometry with a FACSCalibur (Becton Dickinson) counting 6,000 cells per sample.

(ii) Virus binding assay. Cells were prepared in buffer A as above and incubated with purified FMDV O1K-cad^2 (at the indicated concentration) for 1 h on ice, followed by the anti-FMDV MAb C9 (10 μg/ml) and a goat anti-mouse IgG2a-specific, *R*-phycoerythrin conjugate. Virus binding in the presence of manganese was carried out as described above using buffer A supplemented with 1 mM $MnCl_2$ (buffer B). Virus binding in the presence of the activating anti-β1 antibody (MAb 9EG7; 10 μg/ml) was carried out in buffer B. Background fluorescence was determined under three conditions: in the absence of the virus, in the absence of the anti-FMDV MAb, and by incubating the cells with MAb 9EG7 followed by the goat anti-mouse IgG2a-specific, *R*-phycoerythrin-conjugated antibody. All conditions gave nearly identical results, which are shown as a single histogram on the figures.

(iii) Competition experiments. For experiments where integrin-specific antibodies or RGD peptides were used to block binding of FMDV, these reagents were added to the cells in duplicate wells for 0.5 h on ice before the addition of virus for a further 0.5 h. Experiments using cells expressing αvβ1 or the α5/αv(F1-G223)/β1 chimera were carried out in the presence of manganese. Cell-bound virus was detected by using an anti-FMDV MAb as above. When the competing antibody was a mouse IgG2a (e.g., 10D5), virus was detected by using the anti-FMDV MAb B2 (IgG1) followed by a goat anti-mouse IgG1-specific, *R*-phycoerythrin-conjugated antibody. When the competing antibody was a mouse IgG1 (e.g., L230), virus was detected by using the anti-FMDV MAb C9 (IgG2a) followed by a goat anti-mouse IgG2a-specific, *R*-phycoerythrin-conjugated antibody. Background fluorescence was determined for each of the competing MAbs separately by incubating cells with the anti-integrin MAb (100 μg/ml), followed by the anti-isotype-specific conjugated antibody used to detect virus binding.

Infectious center assay. (i) Standard assay. Cells were harvested with trypsin, resuspended in cell culture media, and placed at 37°C for 1 h with continuous rotation. One million cells were resuspended in Tris-buffered saline (pH 7.4) containing the divalent cations as indicated on the figures in the presence or absence of MAb 9EG7 and infected with FMDV O1Kcad^2 or O1BFS (MOI, 0.5) at 37°C for 0.5 h with continuous rotation. Following infection, virus that remained on the outsides of the cells was inactivated by the addition of 1 ml of 0.1 M citric acid buffer (pH 5.2) for 1 min. The cells were washed with PBS (pH 7.5) containing 2 mM $CaCl_2$ and 1 mM $MgCl_2$ and resuspended in 300 μl of the same buffer supplemented with 0.5% fetal calf serum. Dilutions of the infected cells (100 μl) were layered onto subconfluent monolayers of BHK cells as previously described (24), and the monolayers were incubated at 37°C for 40 to 48 h. Infectious centers were visualized as plaques by staining with methylene blue–4% formaldehyde in phosphate-buffered saline (pH 7.5).

(ii) Competition experiments. Anti-integrin antibodies and peptides were added to the cells for 0.5 h on ice prior to the addition of virus, and incubation continued on ice for a further 0.5 h. The cells were washed with cold Dulbecco's minimal essential medium, resuspended in prewarmed cell culture media, and incubated at 37°C for 0.5 h with continuous rotation. Following infection, virus that remained on the outsides of the cells was acid inactivated and the cells were plated onto BHK monolayers as described above.

RESULTS

CHO cells normally express two RGD-binding integrins, αvβ5 and α5β1, but are nonpermissive for field strains of FMDV (21, 32, 42, 54). However, CHO cells are susceptible to infection by FMDV strains that have been adapted for growth in cultured cells and use heparan sulfate proteoglycans as receptors without the mediation of integrins (15, 21, 42, 46). These observations indicate that the failure of FMDV field strains to infect CHO cells results from a lack of an appropriate integrin receptor and not from intracellular deficiencies in virus replication. The CHO variant cell line, CHOB2, lacks endogenous α5. When transfected with human αv cDNA, these cells differ from wt CHO cells in that they no longer express α5β1 but do express αvβ1 (human-αv/hamster-β1) at the cell surface as a functional heterodimer (38, 51, 60). We have used these cells (38) to determine whether αvβ1 has the ability to serve as a receptor for FMDV, the rationale being that these cells express only two αv integrins, αvβ1 and αvβ5, and we and others have previously found that αvβ5 does not appear to mediate infection by FMDV (24, 32, 42). In this study, we compared CHOB2 cells expressing αvβ1 (CHOB2-αvβ1) with untransfected cells. CHOB2 cells expressing αv/α5 chimeras, paired with the endogenous hamster β1 subunit [αv/α5(F1-G232)/β1 and α5/αv(F1-G223)/β1 (see Materials and Methods)] were also included in these investigations. The αv/α5(F1-G232)/β1 chimera has a ligand binding specificity identical to that of wt α5β1 (38) and therefore, like wt α5β1, would not be expected to mediate FMDV infection, whereas the α5/αv(F1-G223)/β1 chimera has a ligand binding specificity identical to that of wt αvβ1 (38). These cells have been reported to express the chimeric integrins at a level similar to that of wt αvβ1 on CHOB2-αvβ1 (38). We also included CHOB2 cells transfected with the wt human β6 subunit that express αvβ6 (CHOB2-αvβ6) (54). Initially, we confirmed by flow cytometry the reported integrin expression profiles for the above cells by using the anti-integrin antibodies listed in Materials and Methods (data not shown).

Next, we determined whether αvβ1 expressed on CHOB2 cells could support FMDV binding. Since integrin-ligand interactions are dependent on divalent cations, initial experiments were carried out in the presence of physiological concentrations of calcium (Ca) and magnesium (Mg). Figure 1 shows that when Ca and Mg were the supporting cations, virus binding was not detected with the parental CHOB2 cells or cells expressing the αv/α5(F1-G232)/β1 chimera. A small amount of virus binding was observed with cells expressing αvβ1 and the α5/αv(F1-G223)/β1 chimera, and virus binding to cells expressing αvβ6 was readily detected.

Since manganese (Mn) ions are known to enhance ligand binding to several integrins (22, 23, 37, 50), we next determined the effect of Mn on virus binding to the integrin-transfected cells. As with Ca and Mg alone, virus binding in the presence of Mn was not detected with untransfected CHOB2 and cells expressing the αv/α5(F1-G232)/β1 chimera (Fig. 1). In addition, Mn did not enhance virus binding to cells expressing αvβ6 over that observed in the presence of Ca and Mg alone. How-

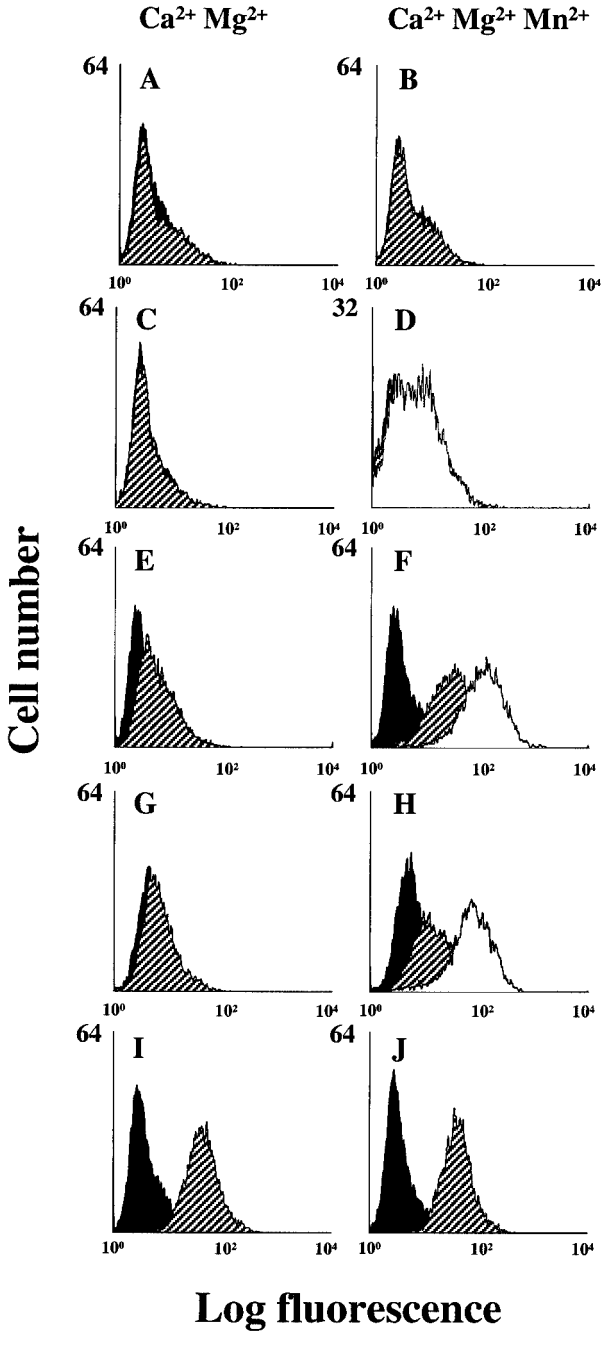

FIG. 1. Flow cytometric analysis of FMDV binding to CHOB2 and integrin-transfected CHOB2 cell lines. FMDV strain O1K-cad[2] (20 μg/ml) was bound to CHOB2 (A and B), αv/α5(F1-G232)/β1 (C and D), CHOB2-αvβ1 (E and F), α5/αv(F1-G223)/β1 (G and H), and CHOB2-αvβ6 (I and J) in the presence of Ca and Mg alone (A, C, E, G, and I) or in the presence of Ca, Mg, and Mn (B, D, F, H, and J). Virus binding (hatched histogram) was determined by using the anti-FMDV MAb C9 and a goat anti-mouse IgG2a-specific *R*-phycoerythrin conjugate. Background fluorescence (black histogram) was determined as described in Materials and Methods. Virus binding in the presence of the activating anti-β1-MAb 9EG7 (D, F, and H) is shown as the open histogram. One experiment representative of three is shown.

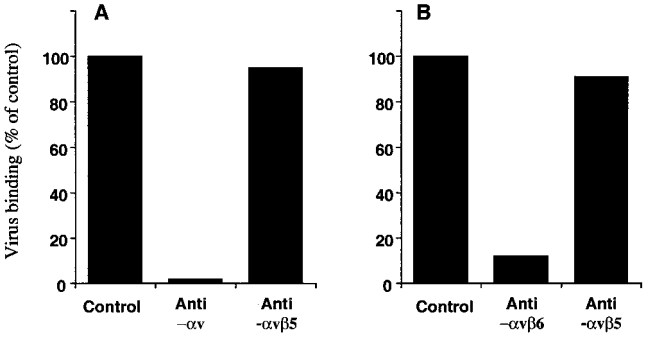

FIG. 2. Anti-integrin MAbs inhibit binding of FMDV to CHOB2 cell lines expressing αvβ1 or αvβ6. CHOB2-αvβ1 (A) or CHOB2-αvβ6 (B) cells were pretreated with the anti-αv MAb, L230 (A), the anti-αvβ6 MAb, 10D5 (B), or the anti-αvβ5 MAb, P1F6 (A and B), at 100 μg/ml for 0.5 h prior to the addition of virus (O1Kcad[2]; 20 μg/ml). Virus binding to cells expressing αvβ1 was carried out in the presence of manganese. Virus binding was detected by flow cytometry as described in Materials and Methods and is expressed as the percentage of virus bound to cells pretreated with assay buffer alone (control). The means from two independent experiments are shown, and in each case the range of observations was within 5% of the mean.

ever, addition of Mn dramatically enhanced virus binding to cells expressing wt αvβ1 and the α5/αv(F1-G223)/β1 chimera (Fig 1). These observations suggest that αvβ1 is expressed in a low-affinity state on the transfected cells and that integrin activation is required for FMDV binding.

Binding to β1 integrins can also be enhanced by activating anti-β1 antibodies (22, 39). We therefore examined the effects of one such antibody, 9EG7 (2), on virus binding. Figure 1 shows that in the presence of 9EG7, virus binding to cells expressing either wt αvβ1 or the α5/αv(F1-G223)/β1 chimera was further enhanced over that in the presence of Mn, whereas virus binding to cells expressing the αv/α5(F1-G232)/β1 chimera was not stimulated by this antibody. These observations confirm that activation of β1 integrins leads to enhanced binding of FMDV to CHOB2-αvβ1.

To verify that FMDV was binding to αvβ1 on the transfected cells, and through an authentic RGD-dependent interaction, we carried out competition experiments with function-blocking anti-integrin MAbs and RGD-containing peptides. Figure 2 shows that virus binding to CHOB2-αvβ1 cells in the presence of Mn was inhibited by the anti-αv MAb L230 but not by the anti-αvβ5 MAb (P1F6). These data demonstrate that an αv integrin is the major site for virus attachment on the αvβ1-expressing cells and that the endogenous hamster αvβ5 does not significantly contribute to virus attachment. We were unable to perform competition experiments using a functional blocking MAb for the β1 subunit since we have not been able to identify such MAbs cross-reactive for hamster β1. However, given that these cells express αvβ1 and αvβ5 as their only RGD-binding integrins (38) and that virus binding was not significantly inhibited by the anti-αvβ5 MAb which is known to be cross-reactive for hamster αvβ5 (54), we conclude that αvβ1 is the major receptor for FMDV attachment on CHOB2-αvβ1. This conclusion is supported by the fact that the anti-αv MAb (L230), which blocks virus binding efficiently, does not recognize the hamster αv subunit, implying that the human αv in the αvβ1 population mediated virus binding.

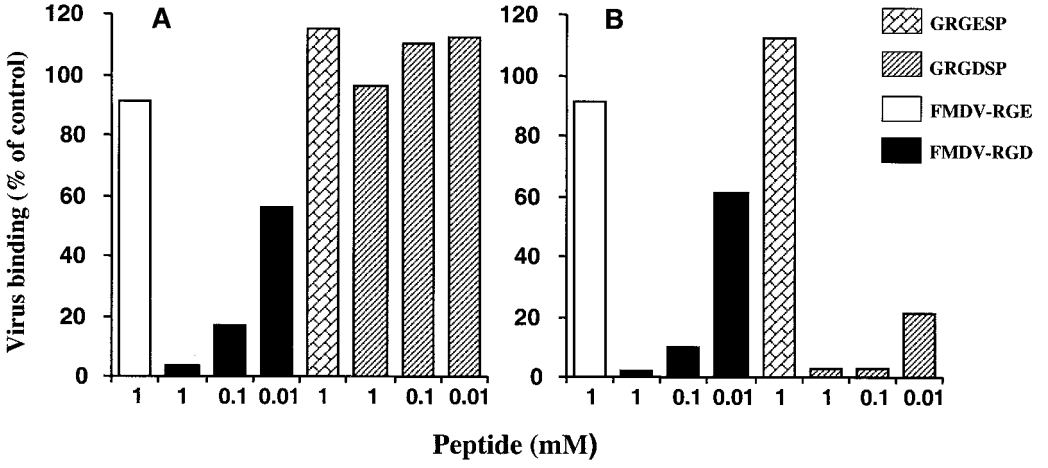

FIG. 3. RGD peptides differentially inhibit FMDV binding to CHOB2 cell lines expressing αvβ1 or αvβ6. CHOB2-αvβ6 (A) or CHOB2-αvβ1 (B) cells were pretreated with RGD or control RGE peptides at the indicated concentrations for 0.5 h prior to the addition of virus (O1Kcad[2]; 20 μg/ml). FMDV-RGD (VPNLRGDLQVLA) has its sequence derived from the FMDV RGD site. Virus binding to cells expressing αvβ1 was carried out in the presence of manganese. Virus binding was detected by flow cytometry as described in Materials and Methods and is expressed as the percentage of virus bound to cells pretreated with assay buffer alone (control). The means from two independent experiments are shown, and in each case the range of observations was within 10% of the mean.

Virus binding to CHOB2-αvβ1 was also inhibited by RGD-containing peptides (Fig. 3). Both a GRGDSP peptide and a longer RGD peptide (FMDV-RGD), with its sequence derived from the FMDV RGD site (see Materials and Methods), were found to inhibit virus binding in a concentration-dependent manner, whereas the control RGE versions of these peptides had only minimal effects on binding. Virus binding to CHOB2-αvβ1 in the presence of the activating anti-β1 MAb 9EG7 was also inhibited by MAb L230 and the RGD peptides but not by MAb P1F6 or the control RGE peptides (data not shown), indicating that following integrin activation, virus binding to these cells was also mediated by αvβ1. Similarly, virus binding in the presence of Mn to cells expressing the α5/αv(F1-G223)/β1 chimera was also specifically inhibited by these reagents (data not shown).

We also compared the abilities of peptides to block αvβ6. Consistent with our previous observations gleaned from experiments using transfected SW480 cells expressing αvβ6, FMDV binding to CHOB2 cells expressing αvβ6 was inhibited by the anti-αvβ6 MAb (10D5) and the FMDV RGD peptide (FMDV-RGD). However, in contrast to the cells expressing αvβ1, the short GRGDSP peptide had little or no effect on virus binding to αvβ6, even when used at high concentrations (Fig. 3). These observations suggest that the residues flanking the RGD tripeptide in the GH loop of VP1 may be required for high-affinity binding to αvβ6. This observation was not unique to the hamster-αv/human-β6 receptor expressed on CHOB2-αvβ6 cells since the same observations were made with SW480 cells expressing human αvβ6 (data not shown).

The above data show that αvβ1 expressed on transfected CHOB2 cells serves as a receptor for FMDV attachment. Next, we determined whether αvβ1 could mediate infection using an infectious center assay. Table 1 shows that for parental CHOB2 or cells expressing the αv/α5(F1-G232)/β1 chimera, only a small number of infectious centers resulted from infection in the presence of Ca and Mg compared to the number observed for cells infected at 4°C. In addition, Table 1 shows that, consistent with the observation that Mn ions did not enhance virus binding, infection of these cells was not significantly enhanced by the addition of Mn. In contrast, infection of cells expressing wt αvβ1 or the α5/αv(F1-G223)/β1 chimera resulted in substantially (≈60 times) more infectious centers than those obtained with the parental CHOB2 cells (Table 1). Furthermore, upon integrin activation, either by Mn ions or by the activating anti-β1 MAb (9EG7), the number of infectious centers observed for these cells was further increased (≈380 or ≈950 times, respectively) over the number obtained with untransfected cells. Consistent with the observation that Mn ions

TABLE 1. FMDV infection of integrin-transfected CHOB2 cell lines

Cells[b]	Virus	Mean no. of infectious centers[a]		
		Ca^{2+} Mg^{2+}[c]	Ca^{2+} Mg^{2+} Mn^{2+}[d]	+ 9EG7[e]
CHOB2	O1Kcad[2]	28	55	ND[f]
αv/α5(F1-G232)/β1		150	500	ND
α5/αv(F1-G223)/β1		1,625	14,750	20,600
CHOB2-αvβ1		1,980	20,900	53,830
CHOB2-αvβ6		420,000	410,000	ND
CHOB2	O1BFS[g]	92,500	ND	ND
CHOB2-αvβ1		105,000	ND	ND

[a] For two experiments carried out in duplicate. Each experiment gave similar results. The number of infectious centers following infection of CHOB2, CHOB2-αvβ6, and CHOB2-αvβ1 at 4°C by O1Kcad[2] was less than 10 infectious centers per 10^6 cells infected.

[b] Cells (10^6) were infected at MOIs of 0.5 PFU/cell.

[c] Infection was carried out in the presence of 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$.

[d] Infection was carried out in the presence of 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, and 1 mM $MnCl_2$.

[e] Infection was carried out in the presence of the activating anti-β1 MAb 9EG7.

[f] ND, not done.

[g] FMDV O1BFS uses heparan sulfate proteoglycans as its cellular receptor.

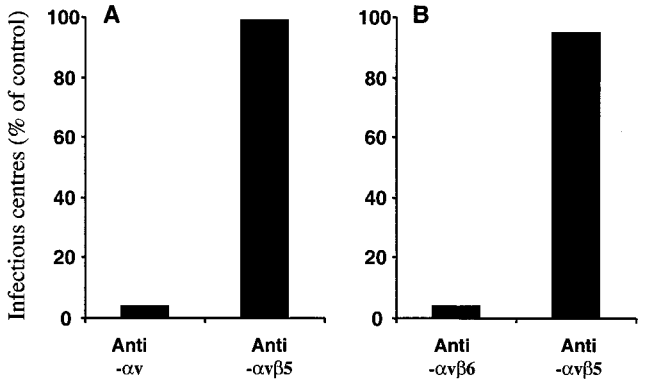

FIG. 4. Infection of integrin-transfected CHOB2 cells is inhibited by anti-integrin antibodies. Duplicate aliquots of CHOB2-αvβ1 (A) or CHO-αvβ6 (B) cells were pretreated with the anti-αv MAb (L230) (A), the anti-αvβ6 MAb (10D5) (B), or the anti-αvβ5 MAb (P1F6) (A and B) at 50 μg/ml for 0.5 h prior to the addition of cold virus (O1Kcad[2]) at a MOI of 1 PFU/cell for a further 0.5 h. The cells were washed to remove unbound virus, and infection was initiated by incubation at 37°C for 0.5 h. Virus that remained on the outsides of the cells was acid inactivated, and the infected cells were used in an infectious center assay. The infection of cells expressing αvβ1 was carried out in the presence of manganese. Control samples were incubated with assay buffer alone (control) before the addition of virus. The means from two independent experiments are shown, and in each case the range of observations was within 5% of the mean.

did not enhance virus binding to αvβ6 (Fig. 1), infection of the CHOB2-αvβ6 cell line in the presence of Mn was not enhanced over that in the presence of Ca and Mg alone (Table 1). Table 1 also shows that CHOB2 cells are permissive for a heparan sulfate-binding strain of FMDV (O1BFS), indicating that as for wt CHO cells, the failure of CHOB2 cells to support infection by field strains of FMDV does not result from intracellular deficiencies in virus replication.

Figures 4 and 5 show that the inhibitory effects of the anti-αv MAb (L230) and the RGD-containing peptides on infection correlated with the ability of these reagents to inhibit virus binding to αvβ1. Thus, in the presence of Ca, Mg, and Mn, the anti-αv MAb (Fig. 4) and the RGD-containing peptides (Fig. 5) were found to specifically inhibit infection of CHOB2-αvβ1. Similarly, infection of these cells in Ca and Mg alone, or in the presence of MAb 9EG7, and infection of cells expressing the α5/αv-β1 chimera in the presence of Ca, Mg, and Mn were also inhibited by MAb L230 and the RGD peptides but not by the anti-αvβ5 MAb P1F6 or the RGE control peptides (data not shown). Figure 4 also shows that infection of CHOB2-αvβ6 was inhibited by the anti-αvβ6 MAb 10D5 but, again, not by P1F6 (ant-αvβ5). Consistent with the observation that the GRGDSP peptide was ineffective at inhibiting virus binding to αvβ6, this reagent did not significantly inhibit infection of CHOB2-αvβ6 cells under conditions where the FMDV-derived peptide (FMDV-RGD) inhibited infection in a concentration-dependent manner (Fig. 5).

DISCUSSION

Several viruses have been reported to utilize multiple RGD-dependent integrins to initiate infection (see the introduction). Prior to these studies, FMDV was reported to use two αv integrins, αvβ3 and αvβ6, as cellular receptors (4, 24). In this study we show that another αv-integrin, αvβ1, also serves as a receptor for FMDV. The main pieces of evidence in support of this finding are as follows. (i) CHOB2 cells, which are normally nonpermissive for field strains of FMDV, become susceptible to infection upon transfection with the integrin αv-subunit and expression of αvβ1 at the cell surface. (ii) αvβ1 serves as the major receptor for virus attachment on the transfected cells, since virus binding is inhibited >98% by a function-blocking MAb that specifically recognizes human αv. (iii) Consistent with the above observations, infection of the transfected cells is also inhibited >98% by the same antibody. In addition, RGD-containing peptides were shown to specifically inhibit virus attachment and infection mediated by αvβ1. Consistent with these data, we found that an α5/αv-β1 chimera (α5/αv(F1-G223)/β1), which has a ligand binding specificity identical to

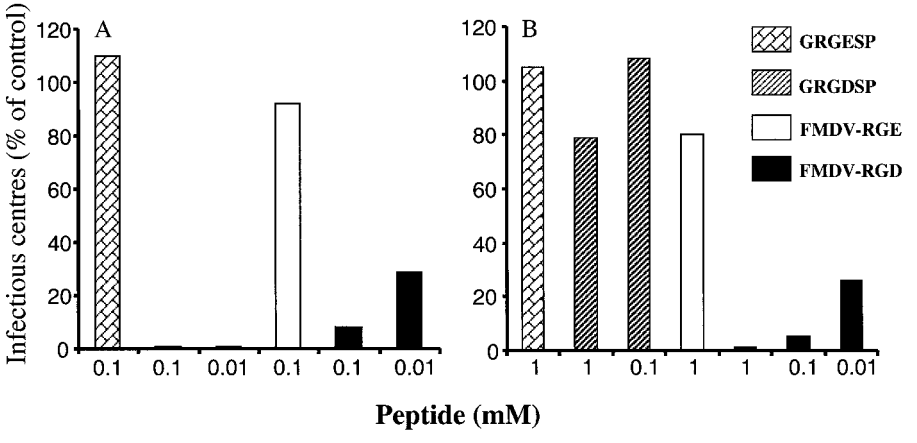

FIG. 5. Infection of integrin-transfected CHOB2 cells is inhibited by RGD peptides. Duplicate cell aliquots of CHOB2-αvβ1 (A) or CHO-αvβ6 (B) were pretreated with RGD peptides at the indicated concentrations for 0.5 h prior to the addition of cold virus (O1Kcad[2]) at a MOI of 1 PFU/cell for a further 0.5 h. The cells were then treated as described for Fig. 4. Infection of cells expressing αvβ1 was carried out in the presence of manganese. Control samples were incubated with assay buffer alone (control) before the addition of virus. The means from two independent experiments are shown, and in each case the range of observations was within 10% of the mean.

that of wt αvβ1, also binds and mediates infection by FMDV, thus providing further evidence for the receptor role of αvβ1. In contrast, an α5/αv-β1 chimera (αv/α5(F1-G232)/β1) with a ligand binding specificity identical to that of wt α5β1 did not support either of these processes, consistent with the observation that α5β1 does not mediate infection by FMDV (24, 32, 42). While these studies were in progress, the crystal structure of the extracellular domains of αvβ3 was reported (58) and reveals that the putative RGD-binding site includes loop regions that lie within residues 1 to 223 of the αv chain, consistent with the results of the present study.

An important finding of our studies is that in the presence of physiological concentrations of Ca and Mg, FMDV binding and infection mediated by αvβ1 are relatively inefficient; however, following integrin activation by Mn or an activating anti-β1 antibody, the ability of αvβ1 to function as a receptor for FMDV is dramatically enhanced. Our data with FMDV are consistent with binding of the natural ligands of αvβ1, which is known to be differentially regulated by divalent cations. Thus, Mn and Mg, but not Ca, support ligand binding and Ca abolishes Mg-promoted adhesion (5, 25, 31, 40, 53). Moreover, activation by an activating anti-β1 MAb similar to that used in the present study overrides the inhibitory effect of Ca on binding of osteopontin to αvβ1 (18). In contrast to αvβ1, αvβ6 on transfected CHOB2 cells appeared to be expressed in a high-affinity state, since neither virus binding nor infection was enhanced by Mn, suggesting that different molecular mechanisms regulate the affinities of αvβ1 and αvβ6 for FMDV.

The number of infectious centers obtained with cells expressing αvβ6 was significantly greater than the number obtained with cells expressing αvβ1 (Table 1). Since αvβ1 (human-αv/hamster-β1) and αvβ6 (hamster-αv/human-β6) expressed on the transfected cells do not share a common subunit, we have not been able to reliably determine the relative level of expression of the transfected integrins. However, some clues regarding the relative efficiency of αvβ1 and αvβ6 at mediating infection by FMDV can be gained by comparing the amount of virus binding with the level of infection. The number of infectious centers obtained with cells expressing αvβ6 was approximately eight times greater than with cells expressing αvβ1, even though the two sets of cells bound similar amounts of virus (Table 1 and Fig. 1). These data suggest that virus bound to αvβ6 may be internalized more efficiently than virus bound to αvβ1.

A short RGD-containing peptide (GRGDSP) and a longer peptide with a sequence derived from the RGD site of FMDV were found to inhibit virus binding and infection mediated by αvβ1. We have previously observed that these peptides also inhibit FMDV binding to purified αvβ3 in vitro (23) and for both αvβ1 and αvβ3, the GRGDSP peptide was the more potent inhibitor. In the present study, we observed that under conditions where the FMDV peptide inhibited virus binding and infection mediated by αvβ6, the GRGDSP peptide was largely ineffective. These observations suggest that residues that flank the RGD tripeptide of FMDV may be required for high-affinity ligand binding to αvβ6. In addition to binding αvβ1, LAP-1 has recently been identified as a high-affinity ligand for αvβ6 (41). As was reported previously, FMDV (RGDLXXL) and LAP-1 (RGDLXXI) share a sequence similarity at the residues following the RGD (22, 24). Given this similarity, and given furthermore that a pentapeptide (DLXXL) with a sequence similar to the residues following the FMDV RGD site has recently been shown to inhibit ligand binding to αvβ6 (26), it is interesting to speculate that the conserved leucine residues located at the RGD + 1 and RGD + 4 positions in FMDV may be required for virus binding to αvβ6.

An important question that has yet to be addressed concerns the roles of the various integrin receptors in the pathogenesis of FMDV. FMDV has a strong predisposition for epithelial cells (1, 8, 9, 10, 47, 59). The primary site of virus replication is thought to be the epithelial cells of the upper respiratory tract. During the development of disease, virus is widely disseminated throughout the body, with secondary sites of replication in many epithelial tissues (1, 10, 59). Currently, no information exists regarding integrin expression in the upper respiratory tract of the natural hosts of FMDV. However, studies with other species have shown that αvβ6 and multiple β1 integrins, but not αvβ3, are expressed on mucosal epithelium (6, 7, 12, 17, 35), suggesting that αvβ6 may have a prominent role in infection at these sites. Little is known about the in vivo cell type expression or tissue distribution of αvβ1 since no complex specific antibodies are currently available. We therefore cannot be certain what role, if any, αvβ1 might play in in vivo infections with FMDV. Nonetheless, the results of the present study suggest that the ability of FMDV to infect αvβ1-expressing cells is likely to be highly regulated by the cellular mechanisms that modulate the ligand-binding affinity of β1 integrins.

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